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L13



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<u>L12</u>	sunflower and cotyledo\$	33	<u>L12</u>
<u>L11</u>	sunflower and cotyledon	18	<u>L11</u>
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<u>L9</u>	osmoti\$ and 12	133	<u>L9</u>
<u>L8</u>	(sunflower same cotyledon) and L6	2	<u>L8</u>
<u>L7</u>	(sunflower adj cotyledon) and L6	0	<u>L7</u>
<u>L6</u>	sucrose and L5	146	<u>L6</u>
<u>L5</u>	12 and @py<2000	190	<u>L5</u>
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<u>L3</u>	sucrose and L2	389	<u>L3</u>
<u>L2</u>	cotyledon and L1	527	<u>L2</u>
<u>L1</u>	sunflower and agrobacter\$	1391	Ll

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1. Document ID: AU 2002247122 A1, WO 200264745 A2, US 20020157138 A1

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L13: Entry 1 of 2

File: DWPI

Aug 28, 2002

DERWENT-ACC-NO: 2002-666999

DERWENT-WEEK: 200427

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TITLE: Transforming and regenerating <u>sunflower cotyledons</u> contacted in an Agrobacterium culture, useful for producing <u>sunflower</u> plant and seeds

INVENTOR: BRAR, G; ROBERTS, G; BRAR, GS; ROBERTS, GA

PRIORITY-DATA: 2001US-268209P (February 12, 2001), 2002US-0683765 (February 12,

2002)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 2002247122 A1	August 28, 2002		000	C12N000/00
WO 200264745 A2	August 22, 2002	E	051	C12N000/00
US 20020157138 A1	October 24, 2002		000	A01H005/00

INT-CL (IPC): A01 H 5/00; C12 N 0/00

Full Title	Citation Front	Review Classification	Date Reference	Claims KMMC Draw. De

Document ID: WO 9800557 A2, AU 9731443 A, US 5958745 A, US 20030028917 A1

L13: Entry 2 of 2

File: DWPI

Jan 8, 1998

DERWENT-ACC-NO: 1998-086981

DERWENT-WEEK: 200313

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TITLE: Biosynthesis of poly-beta-hydroxy; butyrate-co -poly-beta-hydroxy; valerate in plants and bacteria - useful as biodegradable plastic for manufacture of bottles, films, coatings and in drug release applications

INVENTOR: CLEMENTE, T E; CONNOR-WARD, D V; FEDELE, M J; FRY, J E; GRUYS, K J; HINCHEE, M A W; HOWE, A R; KISHORE, G M; MITSKY, T A; PADGETTE, S R; ROZMAN, R J; SLATER, S C; STARK, D M; CONNOR-WARD, D; HINCHEE, M A

PRIORITY-DATA: 1996US-0673388 (June 28, 1996), 1996US-0614877 (March 13, 1996), 1996US-0628039 (April 4, 1996), 1999US-0313123 (May 17, 1999), 2001US-0942891 (August 30, 2001)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 9800557 A2	January 8, 1998	E	311	C12N015/82
AU 9731443 A	January 21, 1998		000	C12N015/82
US 5958745 A	September 28, 1999	•	000	A61K039/395
US 20030028917 A1	February 6, 2003		000	A01H005/00

INT-CL (IPC): <u>A01</u> <u>H</u> <u>5/00</u>; <u>A61</u> <u>K</u> <u>39/395</u>; <u>C07</u> <u>K</u> <u>14/195</u>; <u>C12</u> <u>N</u> <u>1/21</u>; <u>C12</u> <u>N</u> <u>5/10</u>; <u>C12</u> <u>N</u> <u>9/00</u>; <u>C12</u> <u>N</u> <u>9/10</u>; <u>C12</u> <u>N</u> <u>15/52</u>; <u>C12</u> <u>N</u> <u>15/53</u>; <u>C12</u> <u>N</u> <u>15/54</u>; <u>C12</u> <u>N</u> <u>15/60</u>; <u>C12</u> <u>N</u> <u>15/82</u>; <u>C12</u> <u>P</u> <u>7/62</u>

Full	Title Citation	Frent	Review	Classification	Date	Reference				Claims	KMC	Draw, De
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L1 154 SUNFLOWER AND AGROBACTE?

=> s l1 and cotyledo?

L2 14 L1 AND COTYLEDO?

=> d 1-14

- L2 ANSWER 1 OF 14 CABA COPYRIGHT 2005 CABI on STN
- AN 2000:138700 CABA
- DN 20001615966
- TI Transgenic Trifolium repens with foliage accumulating the high sulphur protein, sunflower seed albumin
- AU Christiansen, P.; Gibson, J. M.; Moore, A.; Pedersen, C.; Tabe, L.; Larkin, P. J.
- CS DLF-Trifolium, Danish Plant Breeding, H<o>jerupvej 31, DK-4660 Store Heddinge, Denmark.
- SO Transgenic Research, (2000) Vol. 9, No. 2, pp. 103-113. 45 ref. ISSN: 0962-8819
- DT Journal
- LA English
- ED Entered STN: 20001107 Last Updated on STN: 20001107
- L2 ANSWER 2 OF 14 CABA COPYRIGHT 2005 CABI on STN
- AN 1999:136802 CABA
- DN 19991610135
- TI A focusing device for biolistic transformation of sunflower (Helianthus annuus L.) cotyledons
- AU Vischi, M.; Marchetti, S.; Quagliaro, G.; Olivieri, A. M.; Berville, A. [EDITOR]; Tersac, M. [EDITOR]
- CS Dipartimento di Produzione Vegetale e Tecnologie Agrarie, Via delle Scienze 208, 33100 Udine, Italy.
- SO Helia, (1999) Vol. 22, No. 30, pp. 71-80. 16 ref.
 Price: Conference paper; Journal article .
 Meeting Info.: Proceedings of the Fourth European Conference on Sunflower

Meeting Info.: Proceedings of the Fourth European Conference on Sunflower Biotechnology, Montpellier, France, 20-23 October 1998.

- DT Journal
- LA English
- SL Spanish; French
- ED Entered STN: 19991012 Last Updated on STN: 19991012
- L2 ANSWER 3 OF 14 CABA COPYRIGHT 2005 CABI on STN
- AN 1999:64837 CABA
- DN 19991604573
- TI Agrobacterium-mediated transformation of sunflower (Helianthus annuus L.): a simple protocol
- AU Rao, K. S.; Rohini, V. K.
- CS Department of Biochemistry, Indian Institute of Science, Bangalore 560012, India.

- SO Annals of Botany, (1999) Vol. 83, No. 4, pp. 347-354. 27 ref. ISSN: 0305-7364 DTJournal LΑ English Entered STN: 19990511 ED Last Updated on STN: 19990511 ANSWER 4 OF 14 CABA COPYRIGHT 2005 CABI on STN L2AN 96:25060 CABA DN 19961600899 Expression of foreign genes in sunflower (Helianthus annuus L.) ΤI - evaluation of three gene transfer methods Laparra, H.; Burrus, M.; Hunold, R.; Damm, B.; Bravo-Angel, A. M.; ΑU Bronner, R.; Hahne, G.; Cassells, A. C. [EDITOR]; Jones, P. W. [EDITOR] Institut de Biologie Moleculaire des Plantes, Centre Nationale de la CS Recherche Scientifique et Universite Louis Pasteur, 12, Rue du Generale Zimmer, 67084 Strasbourg Cedex, France. SO Euphytica, (1995) Vol. 85, No. 1/3, pp. 63-74. 36 ref. Price: Conference paper; Journal article . Meeting Info.: Eucarpia Genetic Manipulation in Plant Breeding section meeting, held in Cork, Irish Republic, 11-14 September 1994. ISSN: 0014-2336 DTJournal English LА ED Entered STN: 19960216 Last Updated on STN: 19960216 ANSWER 5 OF 14 CABA COPYRIGHT 2005 CABI on STN L2AN 95:94572 CABA 19951604631 DN ΤI Transformation of sunflower (Helianthus annuus L.) following wounding with glass beads Grayburn, W. S.; Vick, B. A. ΑIJ USDA-ARS, Northern Crop Science Laboratory, Fargo, ND 58105, USA. CS SO Plant Cell Reports, (1995) Vol. 14, No. 5, pp. 285-289. 20 ref. ISSN: 0721-7714 DT Journal LΑ English Entered STN: 19950523 Last Updated on STN: 19950523 L2 ANSWER 6 OF 14 CABA COPYRIGHT 2005 CABI on STN AN 95:21827 CABA DN 19941612058 Towards genetic modification of sunflower TIΑU Peerbolte, R.; Dek, G. J.; Remijn, E. Vanderhave Research, PO Box 1, 4410 AA Rilland, The Netherlands. CS Proceedings of the 13th International Sunflower Conference Volume 2, Pisa, SO Italy, 7-11 September 1992, (1992) pp. 1515-1516. === Meeting Info.: Proceedings of the 13th International Sunflower Conference Volume 2, Pisa, Italy, 7-11 September 1992. DTConference Article LΑ English ED Entered STN: 19950201 Last Updated on STN: 19950201 L2 ANSWER 7 OF 14 CABA COPYRIGHT 2005 CABI on STN 95:21813 CABA ANDN 19941612044 TIGene transfer into sunflower (Helianthus annuus L.) Burrus, M.; Damm, B.; Hunold, R.; Laparra, H.; Pignard, A.; Hahne, G. ΑU
- CS Institut de Biologie Moleculaire des Plantes et Universite Louis-Pasteur, 12 rue du General Zimmer, 67084 Strasbourg, France.

- SO Proceedings of the 13th International Sunflower Conference Volume 2, Pisa, Italy, 7-11 September 1992, (1992) pp. 1426-1431. 8 ref.=== Meeting Info.: Proceedings of the 13th International Sunflower Conference Volume 2, Pisa, Italy, 7-11 September 1992.
- DT Conference Article
- LA English
- ED Entered STN: 19950201

Last Updated on STN: 19950201

- L2 ANSWER 8 OF 14 CABA COPYRIGHT 2005 CABI on STN
- AN 95:21626 CABA
- DN 19941611845
- TI Genetic transformation of the genus Helianthus by Agrobacterium tumefaciens
- AU Biasini, M. G.; Pugliesi, C.; Rocca, M.; Fambrini, M.; Baroncelli, S.
- CS Genetics Section, Department of Agricultural Plant Biology, University of Pisa, Italy.
- SO Proceedings of the 13th International Sunflower Conference Volume 2, Pisa, Italy, 7-11 September 1992, (1992) pp. 1400-1407. 16 ref.===

 Meeting Info.: Proceedings of the 13th International Sunflower Conference Volume 2, Pisa, Italy, 7-11 September 1992.
- DT Conference Article
- LA English
- ED Entered STN: 19950201 Last Updated on STN: 19950201
- L2 ANSWER 9 OF 14 CABA COPYRIGHT 2005 CABI on STN
- AN 94:46330 CABA
- DN 19941603900
- TI Cotyledons: an explant for routine regeneration of sunflower plants
- AU Ceriani, M. F.; Hopp, H. E.; Hahne, G.; Escandon, A. S.
- CS Instituto de Biologia Molecular, CICV, INTA-Castelar, CC 77, 1708 Moron, Buenos Aires, Argentina.
- SO Plant and Cell Physiology, (1992) Vol. 33, No. 2, pp. 157-164. 25 ref. ISSN: 0032-0781
- DT Journal
- LA English
- ED Entered STN: 19941101 Last Updated on STN: 19941101
- L2 ANSWER 10 OF 14 CABA COPYRIGHT 2005 CABI on STN
- AN 92:108681 CABA
- DN 19922323210
- TI Axenic culture of the downy mildew fungus Plasmopara halstedii in Agrobacterium rhizogenes-induced roots of sunflower (Helianthus annuus)
- AU Zahka, G. A.; Viranyi, F.
- CS Institut for Sporeplanter, University of Copenhagen, 0. Farimagsgade 2D, 1353 K. Copenhagen, Denmark.
- SO Canadian Journal of Botany, (1991) Vol. 69, No. 12, pp. 2709-2715. 25 ref. ISSN: 0008-4026
- DT Journal
- LA English
- SL French
- ED Entered STN: 19941101 Last Updated on STN: 19941101
- L2 ANSWER 11 OF 14 CABA COPYRIGHT 2005 CABI on STN
- AN 83:68644 CABA
- DN 19831388633
- TI Evidence for the existence of a universal crown-gall tumor initiation enhancer

```
AU
     Bouckaert-Urban, A.-M.; Vendrig, J. C.
CS
     Katholieke Univ., Leuven, Belgium.
     Planta, (1982) Vol. 156, No. 4, pp. 359-363. 4 fig. 13 ref.
SO
     ISSN: 0032-0935
DT
     Journal
LА
     English
     Entered STN: 19941101
ED
     Last Updated on STN: 19941101
L2
     ANSWER 12 OF 14 CABA COPYRIGHT 2005 CABI on STN
     83:68643 CABA
AN
     19831388632
DN
     Influence of a crown-gall tumour initiation enhancer on bacterial
ТT
     attachment to the host plant cell wall
     Bouckaert-Urban, A.-M.; Brouwers, G.; Thoelen, L.; Vendrig, J. C.
ΑU
CS
     Katholieke Univ., Leuven, Belgium.
SO
     Planta, (1982) Vol. 156, No. 4, pp. 364-368. 5 fig. 18 ref.
     ISSN: 0032-0935
DT
     Journal
LΑ
     English
ED
     Entered STN: 19941101
     Last Updated on STN: 19941101
L2
     ANSWER 13 OF 14 CABA COPYRIGHT 2005 CABI on STN
AN
     82:66582 CABA
DN
     19811376953
TI
     The influence of plant growth regulators on crown-gall initiation on
     cotyledonary leaves of Helianthus giganteus L. in vitro
ΑU
     Bouckaert-Urban, A.-M.; Vendrig, J. C.
     Lab. Pl. Physiol., Catholic Univ. Leuven, Belgium.
CS
     Zeitschrift fur Pflanzenphysiologie, (1981) Vol. 103, No. 1, pp. 75-81. 2
SO
     graphs, 1 tab. 30 ref.
DT
     Journal
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     English
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     Entered STN: 19941101
     Last Updated on STN: 19941101
L2
     ANSWER 14 OF 14 CABA COPYRIGHT 2005 CABI on STN
AN
     76:66311 CABA
DN
     19761321681
TI
     Histophotometric DNA measurements in sunflower seedlings, crown
     gall tumours and habituated callus tissues
     Broekaert, D.; Cazier, C.; Parijs, R. Van
ΑU
CS
     Fac. Med., State Univ. Ghent, Belgium.
SO
     Journal of Experimental Botany, (1976) Vol. 27, No. 98, pp. 532-540. 3
     tab.
     ISSN: 0022-0957
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     Journal
    English
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             14 S L1 AND COTYLEDO?
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L2 ANSWER 1 OF 14 CABA COPYRIGHT 2005 CABI on STN

AB With the aim of increasing the rumen-protected level of the sulphur amino acids cysteine and methionine in Trifolium repens, we introduced the coding sequence of the sunflower seed albumin (SSA) into T. repens by Agrobacterium tumefaciens-mediated transformation. The SSA gene was modified such that the protein would be localised to the endoplasmic reticulum (ER). Four different T-DNA constructions, all containing the SSA gene driven by either the promoter of a gene encoding the small subunit of ribulose bisphosphate carboxylase (Rubisco) from Arabidopsis thaliana (Assu), the promoter of the gene encoding the small subunit of Rubisco of Medicago sativa (Lssu), or the Cauliflower Mosaic Virus 35S promoter (CaMV35S), were transferred to T. repens cv. Haifa. Transgenic T0-plants and inter-transgenic hybrids were analysed for the level of SSA accumulation in the leaves by western blotting. The highest observed level of SSA accumulation was 0.1% of total extractable leaf protein. We observed that the promoter had a substantive effect on the level of SSA accumulation with Assu > CaMV35S > Lssu. Results from the inter-transgenic hybrids showed that the capacity to synthesise SSA was inherited. However the level of SSA accumulation in the leaves generally appears not to be additive with extra transgenic loci. During this work, we attempted to improve the efficiency of A. tumefaciens-mediated transformation of T. repens using the SAAT-method (Sonication Assisted Agrobacterium-mediated Transformation) on cotyledons of T. repens. T-DNA transfer was in general not enhanced by sonication compared to traditional A. tumefaciens-mediated transformation. Furthermore, Southern blot analyses of plants regenerated from the same cotyledon after A. tumefaciens treatment and under selection, indicated that multiple shoots were usually derived from the same transformation event. We concluded from these results that only one plant from each A. tumefaciens-treated cotyledon should be taken to avoid transgenic clones.

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L2 ANSWER 2 OF 14 CABA COPYRIGHT 2005 CABI on STN

Several reports indicate sunflower cotyledons as one AB of the most effective explants for plant regeneration but unlike other sunflower tissues this material is not easily transformed by Agrobacterium. Particle bombardment is an important tool for monocot transformation and may be of use in transforming sunflower cotyledonary tissue. Particle delivery systems currently sold commercially have a limited focusing ability whereas cotyledons exhibit a confined regenerable area restricted to the embryonic axes. To overcome this problem a stainless steel focusing device which can be applied to the DuPont/BioRad PDS1000/He particle gun apparatus was constructed. This device was tested in a series of [beta]-glucuronidase expression tests carried out on sunflower cotyledons of cv. HA89 and experimental lines. Endogenous GUS-like activity was abolished by changing the sample buffer and by performing the reaction at 56[deg]C after having pre-equilibrated explants and buffer for 90 min. The device confined and concentrated more than 90% of the transformation events into a ring-shaped area at 5-6 mm from the target centre. Without the device, transformation frequencies were very low and scattered over a much wider surface. On the basis of these data an appropriate arrangement of the cotyledons could markedly improve transformation success particularly if genotypes with a high frequency of shoot regeneration are

- ΔR A simple and reproducible Agrobacterium tumefaciens-mediated transformation system was developed for sunflower variety KBSH-1. The objective was to substantially eliminate the in vitro regeneration component from the transformation protocol. Two-day-old seedlings with one cotyledon detached were infected with Agrobacterium tumefaciens strain LBA 4404/pKIWI105 harbouring genes for [beta]-glucuronidase (GUS) and neomycin phosphotransferase (NPT II). Following co-cultivation, seedlings were grown aseptically for 5 d on a growth regulator-free basal medium supplemented with 250 [micro]q ml-1 cefotaxime (a bacteriostat used to control gram negative bacteria). Seedlings were screened for transient GUS expression and the shoot portions of the putative transformants were utilized for propagation as transgenic plants. The excised shoots that initiated roots following selection were subsequently transferred to a glasshouse. Molecular analysis of transgenic plants confirmed concordance between the presence of foreign genes and enzyme activity. The transformation regime facilitated rapid generation of up to 2% phenotypically normal fertile plants containing functional transgenes. The transmission and integration of the marker genes in the progeny is demonstrated.
- ANSWER 4 OF 14 CABA COPYRIGHT 2005 CABI on STN

 Suitable sunflower tissues and cells of the inbred line HA300B were transformed either by direct gene transfer into protoplasts, particle bombardment, or Agrobacterium co-culture. While all techniques allowed efficient short-term or transient expression of the introduced gene(s) in the respective tissues, stable transformation was only observed after transformation with Agrobacterium. The latter technique was suitable for the production of transgenic callus from seedling cotyledons and occasional shoots with chimaeric expression of the transgene. Detailed analysis of the interaction of Agrobacterium with this explant showed that infection efficiency was critically dependent on the co-culture conditions, and that the preferentially-transformed cells were not the ones competent for regeneration.
- ANSWER 5 OF 14 CABA COPYRIGHT 2005 CABI on STN

 Cotyledons were removed from young seedlings of cv. SDB861206, and the remaining tissue was uniformly wounded by shaking with glass beads. The wounded tissue was then co-cultivated with a hypervirulent strain of Agrobacterium tumefaciens harbouring the binary plasmid pCNL56, which contained the GUS and NPTII genes. Putative transformants were selected on hormone-free B5 medium supplemented with 100 mg kanamycin/litre, produced roots and were transferred to soil. PCR followed by DNA hybridization demonstrated the presence of the GUS gene in total leaf DNA of 6 primary transformants and 2 progeny plants. However, the A. tumefaciens miaA gene was not detected following PCR amplification of total DNA from transformed leaves. GUS activity was demonstrated in 5 of the T2 transgenic plants. Grafting of potentially transformed scions onto untransformed stocks increased the number of seeds obtained plant.
- ANSWER 6 OF 14 CABA COPYRIGHT 2005 CABI on STN

 Agrobacterium tumefaciens strain LBA4404, containing a BIN19 derived plasmid carrying NPT II and GUS genes, was used to transform immature embryos, cotyledons and cotyledon nodes (included to study indirect and direct regeneration). Despite efficient genetic transformation and a high regeneration frequency, only one fully transformed, unviable shoot and a number of chimaeras were obtained.
- L2 ANSWER 7 OF 14 CABA COPYRIGHT 2005 CABI on STN

 AB The efficiency of genetic transformation of genotype Ha300B was compared for the following systems: co-culture of cotyledons with Agrobacterium tumefaciens; particle bombardment of immature embryos in solid culture media; and direct gene transfer in hypocotyl protoplasts using electroporation or PEG. High levels of transient

expression of foreign DNA were reproducible following incubation of protoplasts in PEG and DNA. Transient expression was also observed following bombardment of embryos with DNA-coated gold particles. GUS positive zones in regenerated shoots were detected after culture of protoplasts with Agrobacterium vectors.

- L2 ANSWER 8 OF 14 CABA COPYRIGHT 2005 CABI on STN
- Leaf explants of the interspecific hybrid H. annuus x H. tuberosus and cotyledonary explants of H. annuus inbred line R857 were inoculated with an A. tumefaciens vector carrying the GUS gene under the control of the CaMV 35S promoter and the NPT II gene. On selection medium containing 25 mg kanamycin/litre, leaf explants formed meristematic centres with buds and embryo-like structures, which developed transformed shoots on MS medium without growth regulators at a rate of 4.7% under optimal conditions. Cotyledonary explants formed meristematic centres on selection medium containing 4 mg kinetin, 0.4 mg IAA and 25 mg kanamycin/litre, which produced transformed shoots at a rate of 2.3% on MS medium without growth regulators. Histochemical staining for GUS activity showed that different tissues and organs had been transformed. Integration of foreign (NPT II) DNA into the genomic DNA was confirmed by PCR and DNA hybridization.
- L2 ANSWER 9 OF 14 CABA COPYRIGHT 2005 CABI on STN
- Culture of cotyledon explants of Helianthus annuus line Ha300 on modified MS medium (MS-Ha) containing 1 mg benzyladenine (BA)/litre resulted in the highest percentage direct shoot regeneration, whilst optimum conditions for callus proliferation involved the use of 0.5, 0.75 or 1 mg NAA/litre alone or in combination with low concentrations of BA. Histological analysis of the kinetics of shoot formation revealed the development of abundant meristematic centres in discrete regions of the basal part of the cotyledon. Out of 20 genotypes, comprising 13 commercial inbred lines and 7 hybrids (derived from crosses with H. argophyllus), evaluated for direct shoot regeneration from cotyledon explants under optimum conditions, only 10 responded. Infection experiments using wild-type Agrobacterium tumefaciens B6S3 showed that tumour tissue was restricted to the basal part of the cotyledon.
- L2 ANSWER 10 OF 14 CABA COPYRIGHT 2005 CABI on STN
- P. halstedii was cultured axenically in association with sunflower roots derived from petioles inoculated with A. rhizogenes. An axenic zoosporangial suspension obtained from sporulating cotyledons, was added to A. rhizogenes-induced root pieces on agar or in liquid nutrient medium. Roots showed profuse P. halstedii sporulation after 1 week. A comparative study in infection morphology with excised sunflower roots (not induced by A. rhizogenes) of the same cultivar showed no difference, and both resembled P. halstedii infection in roots from intact plants. The dual-member cultures with A. rhizogenes induced roots were a good source of axenic P. halstedii inoculum, and zoosporangia remained viable for up to 2 months in dual-member cultures maintained at 18-20[deg]C. Noteworthy features were the balanced state of host and parasite and the prolific production of P. halstedii oospores.
- L2 ANSWER 11 OF 14 CABA COPYRIGHT 2005 CABI on STN
- AB Tumour initiation of different dicotyledonous plants inoculated with Agrobacterium tumefaciens B6 was studied in vivo and in vitro. Tumour formation in weakly susceptible plants was strongly enhanced by exogenously applied active extract fractions from highly susceptible sunflower cotyledons [RPP 61, 814]. The susceptible Kalanchoe, tomato and Pinto bean (Phaseolus vulgaris) were shown to contain a tumour enhancer similar to that in sunflower. No such activity was detected in extracts from weakly susceptible plants or crown gall tissues.

- L2 ANSWER 12 OF 14 CABA COPYRIGHT 2005 CABI on STN
- AB The low or high susceptibility of different plant spp. to tumour formation by Agrobacterium tumefaciens was independent of their capacity to cause bacterial cells to adhere to specific sites on the plant cell walls. Attachment properties of cell wall fragments from sunflower cotyledons were age dependent. The tumour initiation enhancer in extracts from highly susceptible plants did not influence bacterial adherence. Attachment, and the step by which the tumour initiation enhancer is involved, clearly differ in the processes leading to the transformation of a normal cell into a tumour cell.
- ANSWER 13 OF 14 CABA COPYRIGHT 2005 CABI on STN

 Tumour initiation in sunflower cotyledons inoculated with Agrobacterium tumefaciens B6 was studied in vitro. Susceptibility depended on physiological age and could be changed by exogenously applied growth regulators, tumour growth being unaffected by such additions. It is concluded that tumour initiation is not controlled by any specific regulator. The use of regulators may result in a hormonal imbalance creating the opt. condition for transformation of cotyledon cells into tumour cells.
- ANSWER 14 OF 14 CABA COPYRIGHT 2005 CABI on STN L2Crown gall (Agrobacterium tumefaciens) tumours of AB sunflowers were studied with special reference to the amount of DNA/nucleus, determined by Feulgen staining and Lison histophotometry. Results showed that all tissues of normal germinated seedlings remain in the diploid state and that crown gall tumours on the hypocotyl consist predominantly of diploid cells. Organ differentiation (root primordia and young roots) occurred in the tumour tissues and enlarged cambial and parenchymatous cells, scattered or grouped in the tumour, could switch from the mitotic to the endomitotic cell cycle. As a result a polyploid amount of DNA (8-C, 16-C) was measured in the nuclei. However the observations do not justify the classification of the tumours as typical teratomas. The tendency towards polyploidization was also demonstrated in habituated sunflower callus cultures by different techniques. The results are discussed in relation to the thesis that nuclear potentialities in vivo are largely reflected in vitro and in the tumour state.<new para>ADDITIONAL ABSTRACT:<new para>Histophotometric data on meristematic nuclei of the plumule and the root tip and on parenchymatous nuclei in the cotyledon, hypocotyl and root are presented for normally-germinated seedlings of sunflowers. The germination of sunflower seeds in the dark involved DNA synthesis, cell division and differentiation in the meristematic tissues and surplus cell elongation in the hypocotyl, root and cotyledon tissues. The diploid state was predominantly present in all tissues.

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